Thermo Scientific MALDI Material

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June 2010
Beauty of MALDI Imaging

Color coded XICs extracted by ImageQuest™

m/z 1252.951
m/z 1321.776
m/z 1293.750

MALDI MS Imaging of mouse brain

ThermoFisher Scientific
Imaging of Similar Mass Neuropeptides in Neuronal Tissue by Enhanced Resolution MALDI MS with an Ion trap – Orbitrap™ Hybrid Instrument

Peter D.E.M. Verhaert¹,³,⁴, Martijn W.H. Pinkse¹, Maria C. Prieto Conaway², Kerstin Strupat²

Differential images of the distribution of peptides with similar molecular masses (peptide ion images extracted at m/z +/- 0.0025 u).

Note that these images demonstrate the distributions of single isotopes; i.e., the 1011.410 molecular species is clearly resolved from the second isotopes of 1010.472 and 1010.590 (resp. 1011.472 and 1011.590) [see Note 7].

The higher relative intensity of the 1011.410 signal vs the 1010.472 signal is in good agreement with previously detected by different methods variety in abundances of these peptides (Pea-CAH-I is more than twice as abundant as Pea-CAH-II, 12) [see also Note 9 on relative quantification].
Princeton’s Cristea Using Mass Spec to Study How Viruses Manipulate Hosts

September 18, 2008

Name: Ileana Cristea
Position: Assistant professor in molecular biology, Princeton

Background: PhD, University of Manchester, 2002; post-doc, Mass Spectrometry and Gaseous Ion Chemistry at Rockefeller University

Ileana Cristea, an assistant professor in molecular biology, uses mass spectrometry to study chromatin and its modulation.

Earlier this month, she was named one of three recipients of Drug Abuse’s new Avant-Garde awards, given to researchers who are breaking new ground in the prevention and treatment of drug abuse.

As part of that award, Cristea will receive $600,000 each year to continue her research, titled “Proteomic tools to uncover the role of chromatin in the control of gene expression.”

Cristea also recently began research on virus-host protein interactions using Fisher Scientific’s MALDI LTQ Orbitrap XL instrument.

Princeton University Selects Thermo Scientific MALDI LTQ Orbitrap to Enable Proteomics and Genomics Research on Viruses

Thermo Fisher Scientific Inc. has announced that Princeton University is utilizing the MALDI LTQ Orbitrap XL hybrid mass spectrometer for research on virus-host protein interactions in the laboratory of Ileana Cristea, Ph.D.

Dr. Cristea’s work on chromatin and its modulation by viruses promises to further scientists’ understanding about how viruses manipulate their host to their advantage.

The laboratory employs a multidisciplinary approach, incorporating methodologies from modern proteomics, biochemistry, molecular biology and structural biology to answer cutting-edge questions in the field of virus-host interactions.
Mass spectrometry and proteomics: hitting the mark

Nathan Blow

Mass spectrometry instrumentation has made strides in recent years in terms of dynamic range and sensitivity, putting researchers in a better position to use the technology to tackle the challenges of disease biomarker discovery and validation.

At the American Society for Mass Spectrometry (ASMS) meeting in June 2008, mass spectrometry instrumentation aimed at identification of protein biomarkers—proteins indicative of a disease or disease state—appeared to be a recurring theme among instrument manufacturers.

Waters Corporation in Milford, Massachusetts, USA, introduced a new tandem quadrupole mass spectrometry system, called the Xevo TQ MS, along with an associated software package, both designed to move from the biomarker discovery stage towards the verification and validation of target proteins. Agilent Technologies in Foster City, California, USA, introduced their new 6400 series of triple quadrupole liquid chromatography–mass spectrometry systems with increased sensitivity. “The advantage of doing a validation assay on a tandem quadrupole is the improved dynamic range and sensitivity,” notes James Langridge, director of proteomics business development at Waters Corporation.

Recently, Thermo Scientific added MALDI and electron transfer dissociation (ETD) capabilities to their Orbitrap systems.
• Peptide Mass Fingerprinting after Less Specific In-Gel Proteolysis using MALDI-LTQ-Orbitrap and 4-Chloro-alpha-cyanocinnamic Acid
Dimitrios G. Papasotiriou, Thorsten W. Jaskolla, Stavroula Markoutsa, Dominic Baeumlisberger, Michael Karas, and Bjoern Meyer*, Cluster of Excellence “Macromolecular Complexes”, Institute of Pharmaceutical Chemistry, Goethe-University, D-60438 Frankfurt am Main, Germany
J Proteome Res. 2010 May 7;9(5):2619-29.

• High-Spatial and High-Mass Resolution Imaging of Surface Metabolites of Arabidopsis thaliana by Laser Desorption-Ionization Mass Spectrometry Using Colloidal Silver
Iowa State University

• Mass Spectral Analysis of Neuropeptide Expression and Distribution in the Nervous System of the Lobster Homarus americanus
Ruibing Chen¹, Xiaoyue Jiang¹, Maria C. Prieto Conaway², Iman Mohtashemi², Limei Hui¹, Rosa Viner² and Lingjun Li¹
¹Department of Chemistry and School of Pharmacy, University of Wisconsin, Madison, Wisconsin, ²Thermo Fisher Scientific, San Jose, California

• Essential tactics of tissue preparation and matrix nano-spotting for successful compound imaging mass spectrometry.

• Isotope labeled internal standards (ILIS) as a basis for quality control in clinical studies using plasma samples.
Rezeli M, Végvári A, Marko-Varga G, Laurell T.
• Identification of prostate-specific antigen (PSA) isoforms in complex biological samples utilizing complementary platforms
  Ákos Végvári¹, Melinda Rezeli¹, Charlotte Welinder², Johan Malm³, Hans Lilja³,⁴,⁵,⁶, György Marko-Varga¹ and Thomas Laurell¹
  ¹Division of Clinical Protein Science & Imaging, Dept. of Measurement Technology and Industrial Electrical Engineering, Lund University, BMC C13, SE-221 84 Lund, Sweden
  ²Dept. of Oncology, Clinical Sciences, Lund University, Barngatan 2B, SE-221 85 Lund, Sweden, ³Dept. of Laboratory Medicine, Lund University, Malmö University Hospital, SE-205 02 Malmö, Sweden, ⁴Dept. of Clinical Laboratories, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA, ⁵Dept. of Surgery (Urology), Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA, ⁶Dept. of Medicine (GU-Oncology), Memorial Sloan-Kettering Cancer Center, New York, NY 10021, USA

• Resolving the Composition of Protein Complexes Using a MALDI LTQ Orbitrap
  Yang Luo, Tuo Li, Fang Yu, Tal Kramer and Ileana M. Cristea
  Department of Molecular Biology, Princeton University, Princeton, New Jersey, USA
  *JASMS* 2010, 21, 34-46

• A targeted spatial-temporal proteomic approach implicates multiple cellular trafficking pathways in human cytomegalovirus virion maturation.
  Moorman NJ Sharon-Friling R Shenk T Cristea IM
  Department of Molecular Biology, Princeton University, Princeton, NJ 08540.
• MALDI Produced Ions Inspected with a Linear Ion Trap-Orbitrap Hybrid Mass Analyzer
  Kerstin Strupat1, Viatcheslav Kovtoun2, Huy Bui2, Rosa Viner2, George Stafford2 and Stevan Horning1
  1Thermo Fisher Scientific (Bremen) GmbH, Bremen, Germany, 2Thermo Fisher Scientific San Jose, San Jose, California, USA
  JASMS 2009, 20, 1451-1463

• Sequencing of Single and Double Stranded RNA Oligonucleotides by Acid Hydrolysis and MALDI Mass Spectrometry
  Ute Bahr1, Huseyin Aygun2 and Michael Karas1
  1Cluster of Excellence “Macromolecular Complexes”, Institute of Pharmaceutical Chemistry, University of Frankfurt, 60438 Frankfurt, Germany
  2BioSpring GmbH, 60386 Frankfurt, Germany
  Analytical Chemistry 2009, 81, 3173–3179

• The Benefit of Combining nLC-MALDI-Orbitrap MS Data with nLC-MALDI-TOF/TOF Data for Proteomic Analyses Employing Elastase
  Benjamin Rietschel, Dominic Baumlisberger, Tabiwang N. Arrey, Sandra Bornemann, Marion Rohmer, Malte Schuerken, Michael Karas and Bjoern Meyer
  Cluster of Excellence “Macromolecular Complexes”, Institute for Pharmaceutical Chemistry, Goethe-University, Max-von-Laue-Strasse 9, D-60438 Frankfurt am Main, Germany
  Journal of Proteome Research 2009, 8, 5317–5324

• Imaging of Lipids in Spinal Cord Using Intermediate Pressure Matrix-Assisted Laser Desorption-Linear Ion Trap/Orbitrap MS
  Rachelle R. Landgraf1, Maria C. Prieto Conaway2, Timothy J. Garrett1, Peter W. Stacpoole1 and Richard A. Yost1
  1University of Florida, Gainesville, Florida 32611
  2Thermo Fisher Scientific, San Jose, California 95134
  Analytical Chemistry 2009, 81, 8488-8495
MALDI Technique Combined with High Mass Resolution and Accurate Mass Determination in MS

Inset into Melittin @ m/z 2846 from a single full scan m/z 200 – m/z 4000 acquired at resolving power 100,000 @ m/z 400

http://upload.wikimedia.org/wikipedia/commons/3/30/Drinking_Bee.jpg
MALDI Technique Combined with High Mass Resolution and Accurate MS/MS @ m/z 2846

**FTMS + p MALDI Full ms2 2848.00@hcd44.00 [200.00-3000.00]**

- b9
  - 738.48632
- a6
  - 483.32809
- 398.23920
  - R=35406

**1124.67901** y*8

**1211.70885** y*9

**1324.79887** y*10

**1605.96856** y*13

**1776.07419** y*15

**1877.12315** y*16

**2829.73207** precursor

**2845.76660** + 1.8 ppm

- b8
  - 738.48632
- 398.23920
- 2821.8074
  - a5
  - 370.24426
- 299.17084
- b4
  - 242.18582

**697.42095** y*5

**738.48632** y*6

**808.48886** y*7

**851.57043** y*8

**908.48886** y*9

**960.48886** y*10

**1012.48886** y*11

**av (5 scans) = 100 laser shots**

**acquisition time 9 s**

**220 eV**

**almost complete b- and y- ion**

**series**

**plus additional internal**

**cleavages**

**< 3 ppm mass accuracy**

**external mass calibration**

**ASMS 2008**

TP 069, Moehring et al., MALDI-produced ions analyzed by HCD
The Power of MS² Combined with the Simplicity of MALDI

MALDI LTQ XL™ and MALDI LTQ Orbitrap™
The power of MS² combined with the speed of MALDI

MALDI LTQ Orbitrap™
MALDI technique combined with Orbitrap technology
Site-Specific Identification of 3-Nitrotyrosine and Nitrosocysteine Residues in Peptides Using MALDI Mass Spectrometry

Victor Sharov and Christian Schönheit; University of Kansas School of Pharmacy, Lawrence, KS, USA
Zhiqi Hao, Rosa Viner, Roger Biringer and Andreas Hübner; Thermo Fisher Scientific, San Jose, CA, USA

Introduction

Oxidative post-translational modifications are increasingly considered important, particularly for research associated with aging, cardiovascular disease and environmentally associated inflammation. These modifications involve reactive oxygen and nitrogen species with reactions such as nitration or nitrosylation of specific amino acids.

Nitration of tyrosine residues to form 3-nitrotyrosine serves as a fingerprint indicating oxidative stress. S-nitrosylation is the best established example of redox-based specific signaling through the physiological modification of cysteine residues. Like phosphorylation, S-nitrosylation is reversible and plays a role in control of most or all classes of proteins.

It would be valuable to be able to ‘screen’ proteins for these oxidative modifications. Traditional analysis of

Methods

Protein Nitration

Bovine RNase A was nitrated by reaction with a thirtyfold molar excess of TNM at pH 8 for 20 minutes. The reaction was quenched and covalent dimers removed by size exclusion chromatography. Individually nitrated derivatives were separated from the doubly nitrated derivatives (Tyr 115, 76) by isoelectric focusing. Resultant singly nitrated RNase A was denatured and proteolytically digested for MS analysis.

Cysteine Oxidation

Oxidation of cysteine was performed according to a previous method, with some modifications. The cysteine-containing synthetic peptide, MSRPACPNDKYE was oxidized using SIN-1 in 10 mM phosphate buffer, pH 7.4.
Application Notes 471 and 30151, MALDI LTQ Orbitrap XL

Analysis of Sialylated Glycans with the MALDI LTQ Orbitrap Mass Spectrometer using DHB/N,N-Dimethylaniline Matrix

Julian Sahi1, Sergei Svarid2, Deb Charych3 and Rosa Viner4
1Thermo Fisher Scientific, 2University of Manitoba, 3Hive Prime Pharmaceuticals

Accurate MS and MS\(^n\) Analysis with the Thermo Scientific MALDI LTQ Orbitrap

Kerstin Strupat, Steven Horning, Thomas Moebring, Maria C. Prieto Conaney, Rosa Viner, Vatchevel Kootows, Huy Bui, Nick Ligarian, Justin Bletchrope, George Stafford, Thermo Fisher Scientific

**Key Words**
- MALDI LTQ Orbitrap™
- HCD
  Higher Energy Collisional Dissociation
- PMF
  Peptide Mass Fingerprinting

**Introduction**
Matrix-Assisted Laser Desorption/Ionization (MALDI) is a soft ionization technique used in mass spectrometry, allowing the analysis of biomolecules such as proteins, peptides, oligonucleotides and oligosaccharides, which tend to be fragile and fragment when ionized by more conventional ionization methods. It is most similar in character to electrospray ionization both in relative softness and the ions produced, but MALDI generates this allows the detection of (singly charged) M ions with mass resolution up to 100,000 (48 m/z) 40 accurate mass (1-2 ppm or better) routinely.

**Materials and Methods**
The MALDI source is equipped with a Nitrogen Laser (LTB, Berlin, Germany) which operates at 357.1 nm wavelength, 3 ns pulse duration and 60 Hz repetition frequency.
Beauty of MALDI Imaging

- Mouse brain tissue
- DHB matrix
- Accurate mass information of three compounds extracted in narrow window XICs (red, green and blue) using ImageQuest software
- FTMS scans obtained every 33 um
- DHB2_Bremen_toptop_right_33um.raw

Images are created using ImageQuest™
Application Notes 413, 422

Distribution of Irinotecan in Liver and a Human Tumor Xenograft Model by Tissue Imaging Mass Spectrometry

Maria C. Prieto-Conaway1, Shousong Cao1, Farukh Durani1, Youcef Rustum3, Ping Wang3, Khm Marlar3, Latif Kazim1

1Thermo Fisher Scientific, San Jose, CA, USA; 2Roswell Park Cancer Institute, Dept. of Cancer Biology, Buffalo, NY, USA; 3Roswell Park Cancer Institute, Dept. of Cell Stress Biology, Buffalo, NY, USA

The Distribution of Metabolites of Di-(2-ethylhexyl) Phthalate on a Whole Rat by Imaging MS Using a MALDI Ion Trap

T.A. Snow1, M. Prieto-Conaway2, H. Bui1, W.J. Fasano1, L.A. Manning1
1DuPont Haskell Laboratory, Newark, DE, USA; 2Thermo Fisher Scientific, San Jose, CA, USA

Overview

Purpose: Demonstrate mass spectral imaging in whole rat tissue and compare it to results from quantitative whole-body autoradiography (QWBA) technology.

Methods: Parallel dosing of test animals with radioactive and non-radioactive DEHP. Perform QWBA on the hot animal and MS Imaging analysis on the cold animal and compare results.

Results: The [phthalic acid-H3O+H] fragment ion was most abundant upon fragmentation of DEHP in the ion trap in both solution (in vitro experiment) and in tissue. Monitoring and mapping the MEHP metabolite showed more specific distribution within the tissue, as compared to the parent drug.

Methods

DEHP in solution. Neat DEHP was diluted 1:4 in methanol. Further dilution was done directly in 100 mg/mL 2,5 DHB (50/50 v/v acetonitrile/0.1% TFA), for spotting on to a MALDI stainless steel plate.

Tissue studies. Three male S-D rats were orally dosed daily at 300 mg/kg for 14 days and sacked on day 17. One rat was dosed with 14C-labeled DEHP, another with non-labeled DEHP and a third control rat was similarly dosed with corn oil vehicle to compare any differences in the levels of the three metabolites. The rats were frozen in a dry ice/hexane bath and stored at -80°C before cryotome sectioning. Twenty μm thick tissue samples were deposited on acetate film tape and divided into posterior and anterior

ssels has been shown to offer an therapy in human head and as! Two types of tumor xenografts, th high vascular and avascular were used in the above referenced of our study is: that the MALDI LTQ XL is ideally tissue analysis of drugs in tissue; and g capabilities for looking at regions le/inaccessible to the drug in the tumor blish MALDI MS-based imaging as a in cancer therapy research.

active metabolites have been (Figure 1) by HPLC separation ray mass spectrometry detection from s;
ImageQuest allows to create XICs of various kinds of MS scans.

Images of fragment ions obtained by MSMS scans can be displayed, too.

FTMS or FTMS² mass information is extracted with accurate mass.

SW creates images in a post-processing manner.
Each ImageQuest file contains the information about the raw file, such as:

<table>
<thead>
<tr>
<th>Items</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>Scan Filter</td>
<td>FTMS + c MALDI Full ms [160.00-500.00]</td>
</tr>
<tr>
<td>X Data Points</td>
<td>107</td>
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<tr>
<td>Y Data Points</td>
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<tr>
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<tr>
<td>Image Y Offset</td>
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</tr>
<tr>
<td>Mass Tolerance</td>
<td>0.005 amu</td>
</tr>
</tbody>
</table>

| Raw File Name        | D:\1_DEMOS\MALDI\09_11                                             |
| Creator ID           | huibui-local                                                        |
| Creation Date        | Wednesday, November 18, 2009 11:32:34 PM                             |
| Acquisition Time     | 399.9 min                                                           |
| Total Number of Scans| 5136                                                                 |
| Sample Name          |                                                                    |
| Comment              |                                                                    |
| Software Version     | 2.5.5 SP1                                                           |
| Instrument Model     | LTQ Orbitrap XL                                                     |

**Scan Header Information**

- **Scan Filter**: FTMS + c MALDI Full ms [160.00-500.00]
- **X Data Points**: 107
- **Y Data Points**: 24
- **Number of Pixels**: 2568
- **Number of Scans**: 2560
- **X Resolution**: 33 um
- **Y Resolution**: 33 um
- **Image Width**: 3498 um
- **Image Height**: 7859 um
- **Image X Offset**: 19438 um
- **Image Y Offset**: 21232 um
- **Mass Tolerance**: 0.005 amu

**Experiment Information**

- **Number of Scan Events**: 3
- **Scan Event Details**:
  - 1: FTMS + c, res=6000 amu, res=500.00 amu
  - 2: FTMS + c, res=4000 amu, res=500.00 amu
- **Lock Masses**:
  - Pos List Name: N/A
  - Neg List Name: (none)
- **Sample Information**:
  - Sample Position: C3

**MalDI Position File Information**

- **MalDI Position File**: D:\1_DEMOS\MALDI\09_11

**Optical Image Information**

- **Optical Image X Offset**: 19083 um
- **Optical Image Y Offset**: 21119 um
- **Optical Image Width**: 4000 um
- **Optical Image Height**: 2000 um

**MS Image Information**

- **MS Image X Offset**: 395 um
- **MS Image Y Offset**: 113 um
- **MS Image Width**: 3448 um
- **MS Image Height**: 7699 um
- **Rotation Angle**: 0.00 degree
- **Tissue Shape**: Rectangle
- **Micro Region**: n/a
- **Reader Step Size**: 30 um
- **Number of Steps**: 2560
Via the menu bar various features are accessible

- Entire screen, with XIC image, optical image, XIC trace in Chromatogram view and corresponding mass spectrum
- XIC only
- Optical image only
- Mass spectrum only
- Information about scan header, position file instrument method and more, see previous slide
- Average functionality to average over a user-definable region of interest
- Zoom in functionality
- Length / width measure capability
Why MALDI with Orbitrap detection?

- Collisional cooling interface Q00
- MALDI ions are collected in Q00 according to a given AGC (pre-scan) determination
- Ion packages meeting the requested number of ions are sent to the linear trap or Orbitrap det.
- A few to some ten laser shots meet a request of 1e6 charges; these are detected as a single FTMS scan in the Orbitrap detector.
- Resolution allow for a mass resolution of 50,000 (FWHM) @ m/z 2846
- MALDI ion production can afford higher laser energies than an axial ToF geometry (no collisional cooling interface) can
- Robust ion production in Q00 allows for high dynamic range
- Accurate mass is preserved also for low abundant compounds
- Matrix cluster “noise” band is reduced/resolved significantly relative to MALDI-ToF technology
- Detected base line stays flat with increasing laser energy
- Dynamic range is preserved with increasing laser energy
- Instrumentation provides narrow isolation capabilities by linear ion trap technology
- Instrumentation provides dissociation techniques CID, PQD, and HCD
- MS^n capability by linear ion trap features
Summary

• Robust MALDI ion production
  Presence of gas allows high ion current and, therefore, high dynamic range
• LTQ Orbitrap allows the same advantages for MALDI as for ESI
  - accurate mass  low ppm
  - high mass resolution  up to 100,000 @ m/z 400
  - high sensitivity  low attomol
  - dynamic range  > 5000, single scan
• Information-rich MS/MS spectra are provided by the complementary fragmentation techniques of CID and HCD
• High Resolution Precursor ion isolation for MS^n experiments
• In-Gel Digest Analysis for Protein ID via PMF and MS/MS
• LC-MALDI Analysis for Protein ID via MS/MS
• In Source Decay MALDI for Top Down IDs and sequencing
• Small Molecule analysis with little matrix interference
• Confident PTM analysis
• ImageQuest visualization SW enabled for highly resolved and mass accurate spectra
Mass Tech’s AP MALDI source applied to Thermo Scientific Exactive MS

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June 2010
AP MALDI on the Exactive MS

AP/MALDI PDF+ Ion Source
Available for the Thermo Scientific Exactive MS

AP/MALDI PDF+ on the Exactive™ MS
- Low femt mole sensitivity
- Mass accuracy in agreement with Exactive specifications
- High resolution up to 100,000
- Easy to operate with reliable results

AP/MALDI PDF+ includes
- Nd:YAG high repetition rate laser
- Software controlled energy attenuation
- Automated image recognition of spot centers

AP/MALDI PDF+ advantages
- High throughput applications
- Improved sensitivity
- Lower lifetime ownership cost

Easily interchangeable with ESI in just minutes!